REMARKS

Claims 1-41 currently appear in this application. The Office Action of January 13, 2005, has been carefully studied. These claims define novel and unobvious subject matter under Sections 102 and 103 of 35 U.S.C., and therefore should be allowed. Applicants respectfully request favorable reconsideration, entry of the present amendment, and formal allowance of the claims.

Election/Restriction

Group II has been elected without traverse. Claim 4 is now withdrawn from this application, and nonelected compounds have been deleted from the remaining claims.

Specification

The disclosure is objected to because the preliminary amendment filed April 14, 2003, includes an amendment to the specification which is not properly identified.

This amendment has been corrected to amend the paragraph starting at line 15 of page 74.

The formulae on pages 9 and 10 have been corrected.

Priority

The priority has been added to the specification in accordance with the Examiner's helpful suggestion.

Rejections under 35 U.S.C. 112

Claims 13-20 and 23-26 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The Examiner alleges that claims 13-20 and 23-26

are directed to a method for treating a disease which is associated with JNK pathway.

This rejection is respectfully traversed. In the Background section of the application, the function of JNK is described in detail at page 2, line 10 through page 4, line 20. Specifically, the specification refers to WO/9849188 which teaches the use of a JNK-interacting protein which has been assayed for overcoming apoptosis related disorders. These polypeptides have been confirmed to have an inhibitory effect on the JNK kinase pathway, although there are a number of drawbacks associated with their use.

The sulfonamides of the present invention are inhibitors of JNK (c-Jun Kinase). It is well known that JNK is involved in inflammatory, autoimmune disorders and cancer. Numerous scientific articles have been published, which article has been referenced in the specification at pages 2-4 of the application as filed.

Submitted herewith is a copy of Anthony M. Manning and Roger J. Davis, "Targeting JNK for Therapeutic Benefit," *Nature* 2003, Vol. 2, pages 554-565. This review article summarizes the general knowledge in the filed of JNK and its involvement in a variety of diseases. Specifically, beginning at page 555, right column, through pager 562, left column, a variety of diseases are described in which JNK plays a role, and, therefore, inhibiting the production of JNK can be used to treat these diseases. The article furthermore reports on JNK inhibitors (*e.g.*, those of Celgene, Vertex, Takeda, Merck and Roche) that are in clinical development for treating these various disorders, as described on pages 562-564. Thus, it is

general knowledge that inflammatory, autoimmune disorders and cancer can be treated by inhibiting JNK.

Claims 1-3 and 5-26 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

This rejection is respectfully traversed. First of all, with respect to "derivatives", this term is not vague, because the sulfonamide derivatives claimed are only those which have the structure recited in the claims. That is, the compounds claimed are derivatives of sulfonamides. However, to simplify the issues, "derivatives" has been replaced with --compounds--. The ranges of limitations have been deleted from the claims and presented in new claims. Claims 1 and 2 have been amended to eliminate non-elected subject matter. Claim 2 has been amended to eliminate use as a medicament and to change "substi-tuted" to -substituted--. Claim 3 has been amended to change "$L^2$are" to --L^2 are—and to correct the language for Markush groups.

Claim 8 has been amended to recite a proper Markush group. With respect to antecedent basis, a thioxo-dihydropyridine is a substituted heteroaryl group, as recited in claim 1.

Claim 10 has been amended to recite a proper Markush group, as well as to correct a self-evident error in the recitation of a C_1-C_{12} alkyl group. The recitation of aryl or heteroaryl substituents are substituted by halogen, hydroxy, nitro, **or** sulfonyl, the alternative language being a proper Markush group.

It is not understood why the species in lines 7-8 of page 17 are a duplicate of the species in lines 9-10 on page 13. That is, N-(4-chlorophenyl)-2-(5{[4-(2-oxo-2,3-dihydro-1H-benzamidiazol-1-yl)piperiodin-1-yl]sulfonyl}thien-2-acyl) acetamide is not the same as the benzamide recited on page 13. It is not understood how the species in lines 15-16 on page 19 are the same as the species in lines 13-14 on page 9 in claim 11.

In claim 11, page 21, 9H-purin-9-yl is a purine ring substituted at position 9.

Claims 13-20 and 23-26 are said to be vague and indefinite in that the claim provides for the use of claimed compounds, but the claim is said not to set forth any steps involved in determining which are the disorders capable of being treated by modulating the activity of JNK.

This rejection is respectfully traversed. As discussed above, one skilled in the art is aware of a number of diseases that respond to inhibiting the activity or abnormal expression of JNK. It is known that immuno- and/or neuronal-related diseases or pathological states in which inhibition of JNK2 or JNK3 plays a critical role such as epilepsy; neurodegenerative diseases including Alzheimer's disease, Huntington's disease, Parkinson's disease; retinal diseases; spinal cord injury; head trauma; autoimmune diseases including multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis; asthma; septic shock; transplant rejection; cancers, including breast, colorectal, pancreatic; and cardiovascular diseases including stroke, cerebral ischemia, arteriosclerosis, myocardial infraction, and myocardial reperfusion injury.

In vitro tests were conducted using 14 compounds according to the present invention to determine if these compounds had any activity in inhibiting JKN2 or JKN3. The results of these tests are shown in the table spanning pages 81 and 82. It was also determined that the compounds' activity was limited to JNK2 and JNK3, as the compounds had virtually no effect on p38 and ERK2. Additionally, a test described on page 81 of the present specification describes how the compounds of the present invention rescue neurons from cell death.

In vivo studies were conducted with compounds of the present invention, and it was demonstrated on pages 88 and 89 that the compounds significantly reduced the level of inflammatory cytokines induced by LPS challenge in mice, and protecting cells from death during a stroke event in gerbils.

The compounds of the present invention have been demonstrated to have considerable activity as inhibitors of JNK2 and JNK3, which are known to be associated with many neuronal and autoimmune conditions and diseases, as well as cardiovascular protection. Thus, the present invention is directed to compounds that inhibit the JNK kinase pathway and therefore can be used to treat diseases mediated by the JNK pathway, as described above.

The compounds of the present invention were subjected to a total of six different assays, as shown in the specification as filed at pages 81-89, namely:

- A JNK2 and 3 *in vitro* assay
- A sympathetic neuron culture and survival assay
- An IL-2 release assay
- A c-Jun reporter assay

- Showing that the LPS induced endotoxin shock in mice
- Global ischemia in gerbils.

In view of the biological data obtained from these assays, there can be no doubt that:

- The compounds claimed herein are JNK inhibitors
- The claimed compounds are useful in rescuing neuronal cells from cell death
- The claimed compounds are suitable for inhibiting pro-inflammatory IL-2 production
- The claimed compounds are suitable for protecting cells from death during a stoke event; and therefore, that

The claimed compounds are suitable for treating neuronal disorders, autoimmune diseases, cancer, and cardiovascular diseases in light of the general knowledge concerning the role of JNK in these disorders.

Therefore, it is respectfully submitted that the present application contains sufficient information and data in support of the utility of the compounds of formula (I) as well as how to use these compounds as required by 35 U.S.C., second paragraph.

One skilled in the art can, without undue experimentation readily determine what is an appropriate dosage of a sulfonamide compound according to the present invention. One skilled in the art has some idea of the degree of JNK pathway that must be inhibited, and the strength of the individual sulfonamide compound. These values can be determined by routine experimentation that is used to

determine the optimum dosage and mode of administration of a particular drug.

Claims 16-19 are said to be vague and indefinite in that it is not known what is meant by "according to said formula I" when formula I is not present in the claim.

It is respectfully submitted that a dependent claim includes all of the limitations of the claim from which it depends. Claim 13, from which claims 16-19 depend, recites formula I, which means that formula I is indeed part of claims 16-19.

Claim 21 has been amended to delete "'" from the claim, and to recite that the definitions of $(R^6)n$, L^1 and L^2 are those of claim 1, from which claim 21 depends.

Claim 22 has been amended to recite that the definitions of Ar^1, Ar^2 and R^1 are those of claim 1, from which claim 22 ultimately depends.

Double Patenting

Claims 1-3 and 5-26 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-19 of copending application No. 10/381,197.

Claims 1-3 and 5-26 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-16 of copending application No. 10/381,200.

Claims 1-3 and 5-26 are provisionally rejected under the judicially created doctrine of obviousness-type double

patenting as being unpatentable over claims 1-19 of copending application No. 10/381,665.

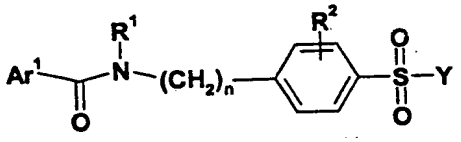
These rejections are respectfully traversed. It should be recalled that the Examiner stated in the Restriction Requirement that compounds of formula (I) in which Y is piperidino are a different invention from compounds in which Y is a piperazine, pyrrolidine, azepan, or diazepine. That is, according to the Examiner, a small structure difference in the cyclic amine Y gives rise to a different invention.

If compounds in which Y is a different moiety are considered to be patentably distinct inventions, the same logic must apply with respect to the compounds claimed in copending applications 10/381,200; 10/381,665 and 10/381,197. The compounds in the cited applications can be distinguished from the compounds claimed herein as follows:

- In 10/381,200, the compound of formula (I) are characterized in that Ar^2 is an aryl or heteroaryl group having at least one hydrophilic substituent. No such compounds are disclosed or even suggested in the present application.
- In 10/381,665, the compounds of formula (I) are characterized in that the 4-12 membered saturated cyclic or bicyclic alkyl Y is substituted with at least one ionizable moiety to which a lipophilic chain is attached. In one embodiment, the ionizable moiety to which a lipophilic chain is attached is the group L^1 is $-NHR_3$, where R^3 is a straight or branched C_4-C_{12} alkyl, preferably a C_6-C_{12} alkyl, optionally

substituted with a cyclohexyl group of a benzyl group. No such compounds are disclosed or suggested in the present application.

In 10/381,197, the compounds of formula 1 are such that Ar^2 is a phenyl moiety:



(I)

The presently claimed compounds are quite different from these compounds.

In view of the above, it is respectfully submitted that the claims are now in condition for allowance, and favorable action thereon is earnestly solicited.

Respectfully submitted,

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TARGETING JNK FOR THERAPEUTIC BENEFIT: FROM JUNK TO GOLD?

Anthony M. Manning and Roger J. Davis‡*

The c-Jun NH_2-terminal kinases (JNKs) phosphorylate and activate members of the activator protein-1 (AP-1) transcription factor family and other cellular factors implicated in regulating altered gene expression, cellular survival and proliferation in response to cytokines and growth factors, noxious stimuli and oncogenic transformation. Because these events are commonly associated with the pathogenesis of a number of human diseases, the potential of JNK inhibitors as therapeutics has attracted considerable interest. Here we discuss the evidence supporting the application of JNK inhibitors in inflammatory, vascular, neurodegenerative, metabolic and oncological diseases in humans, and describe the present status of drug discovery targeting JNK.

AP-1 TRANSCRIPTION FACTOR The transcription factor AP-1 is composed of homo- or hetero-dimers of proteins that belong to the FOS and JUN families. JUN proteins can homo-dimerize, but FOS proteins can only form stable dimers with JUN. AP-1 dimers can be phosphorylated by JNK and other MAP kinases and hence develop an enhanced DNA-binding capacity and transcriptional activity.

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JNK was initially identified and purified by Kyriakis *et al.* as a protein kinase that was activated in the liver of rodents exposed to cycloheximide[1]. Independent studies identified JNK as a stress-activated protein kinase that phosphorylated c-Jun on two sites in the NH_2-terminal activation domain[2,3]. Subsequent studies led to the molecular cloning of JNK[4,5] and the demonstration that it is a member of the mitogen-activated protein kinase (MAPK) group of signalling proteins[6]. Ten JNK isoforms are created by alternative splicing of messenger RNA transcripts derived from three genes: *JNK1*, *JNK2* and *JNK3* (FIG. 1)[7]. Gene disruption studies in mice demonstrate that JNK is essential for tumour-necrosis factor-α (TNF-α)-stimulated c-Jun phosphorylation and AP-1 TRANSCRIPTION FACTOR activity[8], and is also required for some forms of stress-induced apoptosis[9].

Certain cytokines, mitogens, osmotic stress and ultraviolet irradiation activate the JNK pathway, as depicted in FIG. 2. The upstream pathway leading to JNK activation is complex: cell- and stimulus-specific responses that lead to JNK activation are probably controlled by physically distinct intracellular complexes of multiple signalling proteins. JNK activation leads to the phosphorylation of a number of transcription factors — most notably the c-Jun component of AP-1 — and cellular proteins, particularly those associated with apoptosis (for example, Bcl2, p53 and so on).

JNK is activated by dual phosphorylation of the motif Thr-Pro-Tyr located in the activation loop[4]. JNK inactivation can be mediated by serine and tyrosine phosphatases, but also by a family of dual specificity MAP kinase phosphatases[6]. JNK phosphorylation is mediated by two MAPK kinases (MAPKKs) — MAP2K4 (also known as MKK4) and MAP2K7 (also known as MKK7) — that can cooperatively activate JNK. MAP2K4 preferentially phosphorylates JNK on tyrosine, whereas MAP2K7 preferentially phosphorylates JNK on threonine[10]. Gene disruption studies in mice demonstrate that both MAP2K4 and MAP2K7 are required for full activation of JNK by environmental stressors, and that MAP2K7 is essential for JNK activation by TNF[11]. The MAP2K4 and MAP2K7 protein kinases are also activated by dual phosphorylation within the activation loop, and this phosphorylation is mediated by one of a large group of upstream protein kinases, including transforming growth factor-β-activated kinase-1 and members of the MAPK/extracellular-regulated kinase group, the mixed lineage kinase group and the activator of S-phase kinase group of MAPKK kinases (MAPKKKs)[6]. Together, these protein kinases are able to form signalling cascades that can function as defined signalling modules that mediate JNK activation in response to specific stimuli (see FIG. 3).

The specificity of signal transduction by JNK is mediated, in part, by protein–protein interactions[12].

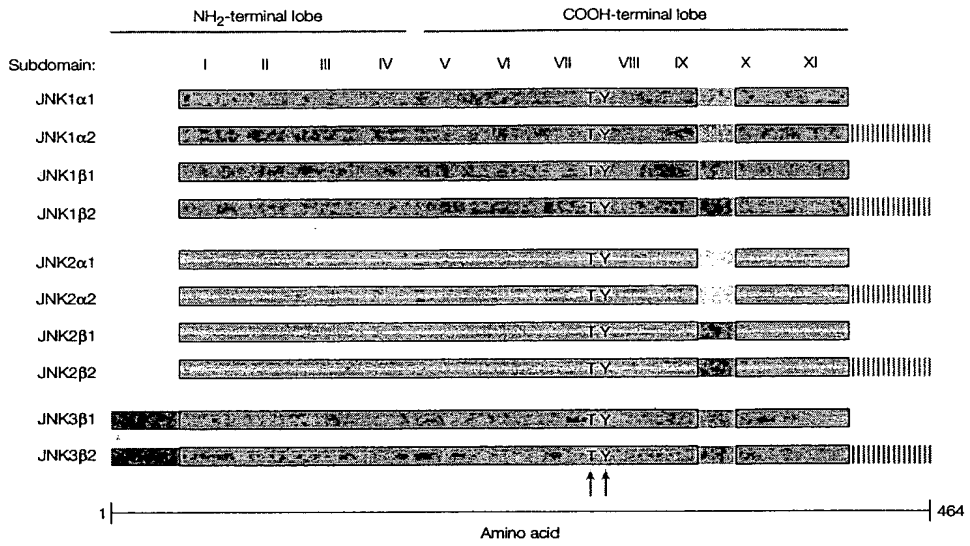

Figure 1 | **Structural features of the JNK proteins.** The c-Jun NH_2-terminal kinases (JNKs) are typical of serine/threonine kinases, comprising 11 protein kinase subdomains (indicated as I–XI). The protein kinase activation loop is located between domains VII and VIII, and contains the threonine (T) and tyrosine (Y) residues that are phosphorylated for full kinase activation. The members of the JNK family are generated by alternative splicing of three JNK genes (*JNK1*, *JNK2* and *JNK3*, depicted as orange, light and dark blue, respectively) to produce ten different isoforms. The differences are indicated by the shaded regions. There are two key alternative splicing sites: the first is between subdomain IX and X of the COOH-terminal lobe of the protein, which results in splice forms that demonstrate altered substrate specificity; the second alternative splicing site occurs at the C terminus of the protein, and results in proteins that differ in length by either 42 or 43 amino acids (depicted as hatched regions).

MAPKs contain a common docking site that is distant from the active site that binds to docking motifs (D-domain and FXFP) that are located in interacting proteins, including substrates, MAPKKs, and MAPK phosphatases[12]. JNK also interacts with scaffold proteins that can assemble functional signalling modules[13]. Examples of scaffold proteins include the JNK-interacting proteins (JIPs) that are transported by the microtubule motor protein kinesin[13]. Such scaffold proteins can regulate localized activation of JNK within cells.

The complexity of the JNK pathway provides multiple opportunities for the design of small-molecule inhibitors that might modulate signalling by the JNK pathway. Each target has both potential benefits and disadvantages for drug design. One approach is to directly target the JNKs, a strategy that is reviewed herein. This strategy is being aggressively pursued by a number of drug discovery companies, as evidenced by the patent and scientific literature. However, owing to the breadth of physiological functions mediated via signalling through the JNK family, direct inhibition at the level of the JNKs could also have liabilities. Alternatively, targeting the upstream MAPKKs or MAPKKKs within the ordered hierarchy of the signalling cascade could offer greater specificity in blocking pathological responses, and we refer the reader to excellent reviews of such agents[14,15].

Inflammatory diseases

Autoimmune and inflammatory diseases arise from inappropriate activation of the immune system, resulting in the overproduction of immune cells, inflammatory cytokines and tissue-destructive enzymes. These cells and proteins attack and destroy healthy tissue, giving rise to a number of diseases such as rheumatoid arthritis, multiple sclerosis, asthma, inflammatory bowel disease and psoriasis, as well as transplant rejection. Although available drugs alleviate many of the symptoms of disease, they generally do not target the underlying mechanisms, are relatively non-selective and have dose-limiting side effects. The search for agents that target the underlying pathogenic mechanisms of these diseases has accelerated during the past decade primarily due to our enhanced knowledge of molecular and genetic pathways regulating the immune system.

Activated immune cells express many genes encoding inflammatory molecules, including cytokines, growth factors, cell surface receptors, cell adhesion molecules and degradative enzymes. Many of these genes, including those encoding TNF-α, interleukin-2 (IL-2), E-selectin and matrix metalloproteinases (MMPs) such as collagenase-1, are regulated by the JNK pathway, through activation of the transcription factors AP-1 and ATF-2 (REF. 16).

Monocytes, tissue macrophages and tissue mast cells are key sources of TNF-α. The JNK pathway regulates

TNF-α production in bacterial lipopolysaccharide-stimulated macrophages, and in mast cells stimulated through the FcεRII receptor[17,18]. Inhibition of JNK activation effectively modulates TNF-α secretion from these cells. MMPs promote cartilage and bone erosion in rheumatoid arthritis, and generalized tissue destruction in other autoimmune diseases. Inducible expression of MMPs, including MMP3 and MMP9, and type II and IV collagenases, are regulated by activation of the JNK pathway and AP-1 (REF. 19). In human rheumatoid synoviocytes activated with TNF-α, IL-1 or Fas ligand, the JNK pathway is activated[20]. Inhibition of JNK activation results in decreased AP-1 activation and collagenase-1 expression. Activated JNK can also be detected in synovial fibroblasts and chondrocytes from the joints of osteoarthritic patients, but not from normal controls, and has been suggested to play a role in the chondrocyte injury and cartilage degeneration that are features of this disease[21,22]. The presence and activity of the JNK pathway in multiple cell types involved in the inflammatory process has drawn attention to the development of JNK inhibitors as immuno-modulatory agents (FIG. 4).

Recently, several studies have reported the effects of the administration of JNK inhibitors in animal models of arthritis and asthma. SP-600125 was reported as a selective inhibitor of JNKs 1, 2 and 3 (IC_{50} = 110–150 nM), but with much less activity against the closely related p38 MAPK (IC_{50} >30 μM)[23]. SP-600125 inhibits IL-1-induced phosphorylation of JNK and c-Jun in cultured synoviocytes from rheumatoid arthritis patients, and inhibits the production of MMP13, a key enzyme associated with cartilage destruction[21]. Administration of SP-600125 inhibits JNK activation and collagenase expression in the joints of rats with adjuvant arthritis. Animals showed a significant reduction in paw swelling and bone and cartilage damage. In light of these findings, the inhibition of JNK could be considered as a

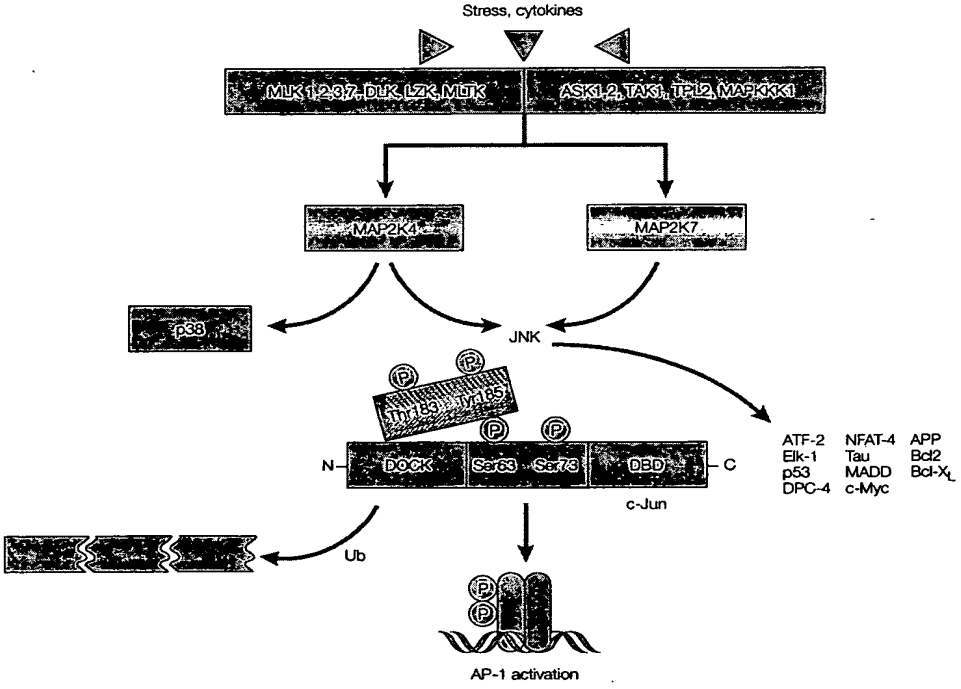

Figure 2 | **Organization of the JNK signal transduction cascade.** The c-Jun NH_2-terminal kinase (JNK) pathway is variably activated in cells by extracellular stimuli including stress and cytokines. A variety of receptor-associated signalling mechanisms lead to the activation of mitogen-activated protein kinase kinase kinases (MAPKKKs) that are capable of activating either MAP kinase kinase 4 or 7 (MAP2K4 or MAP2K7). MAP2K4 can activate either the JNKs or the p38-MAP kinases. MAP2K7 selectively activates the JNKs. JNK activation requires dual phosphorylation of both Thr183 and Tyr185, triggering the specific interaction of activated JNKs with a number of substrates including the c-Jun component of the activator protein-1 (AP-1) transcription factor. Resulting phosphorylation of c-Jun on Ser63 and Ser73 results in the acquisition of enhanced transcriptional activity of complexes containing AP-1. In the absence of serine phosphorylation, c-Jun is degraded by a ubiquitin (Ub)-dependent proteolytic pathway. APP, amyloid precursor protein; ASK, activator of S-phase kinase; ATF, activating transcription factor; Bcl, B-cell lymphoma protein; DBD, DNA-binding domain; DLK, dual leucine zipper kinase; DOCK, docking region; DPC-4, dystrophin-associated protein complex-4; Elk-1, member of the ETS oncogene family; LZK, leucine zipper-bearing kinase; MADD, MAPK-activating death domain; MLK, mixed lineage kinase; MLTK, mixed lineage kinase-related kinase; c-Myc, cellular myelocytomatosis oncogene; NFAT, nuclear factor of activated T cells; TAK, transforming growth factor-β-activated kinase; Tau, microtubule-associated protein tau; TPL2, tumour progression locus 2.

IC_{50}
The concentration of drug at which activity of a particular assay is inhibited by 50%. This is a typically used value to describe the relative potency of a drug agent.

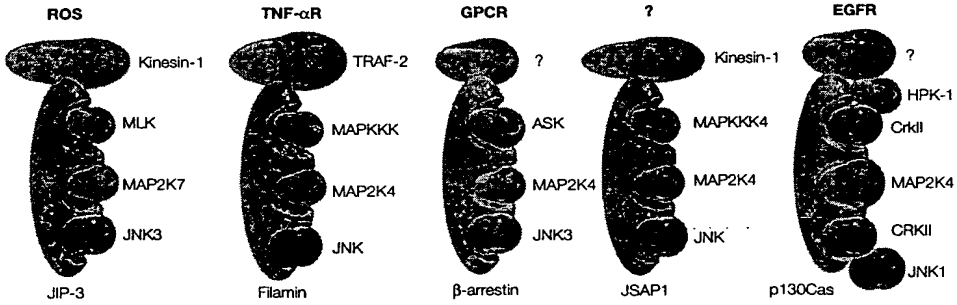

Figure 3 | **Modular organization of JNK signalling complexes.** The protein kinases that form c-Jun NH_2-terminal kinase (JNK) signalling modules in cells do so through interaction with scaffold proteins. Studies in yeast established the concept that scaffold proteins are crucial components of mitogen-activated protein kinase (MAPK) pathways. Five groups of potential scaffold proteins that might coordinate JNK signalling have been reported: JNK-interacting protein (JIP), filamin, β-arrestin, JNK/stress-activated protein kinase-associated protein 1 (JSAP1) and p130Cas. These scaffold proteins bind discrete members of the JNK pathway, and co-localize them within the cell. It seems that these scaffolds provide spatial and stimulus-specific regulation of JNK function. JIP belongs to a group of proteins that were identified as proteins that bind JNK, but were subsequently shown to bind other components of the pathway, including mixed lineage kinase (MLK) and MAPK kinase 7 (MAP2K7). Filamin is a large protein that interacts with and organizes actin filaments, but also binds to MAPKK kinase (MAPKKK), MAP2K4 and JNK. The arrestin group of adapter proteins, including β-arrestin 2, bind to G-protein coupled receptors (GPCRs) following ligand engagement and have important functions in the termination of heterotrimeric G protein activation by GPCRs. Recent studies demonstrate that β-arrestin 2 can bind components of the JNK pathway. p130Cas is a multiprotein complex that includes JNK, recruited through association with v-crk sarcoma virus CT10 oncogene (CRKII). A more detailed review of JNK complexes is presented in REF. 103. ASK, activator of S-phase kinase; EGFR, epidermal growth factor receptor; HPK-1, haematopoietic progenitor kinase-1; ROS, reactive oxygen species; TNF-αR, tumour-necrosis factor-α receptor; TRAF, TNF receptor-associated factor.

potential therapy for rheumatoid arthritis. These studies were extended using JNK2 knockout mice in a model of passive murine collagen-induced arthritis, in which JNK2 was demonstrated to be a key determinant of matrix degradation, but was less important for inflammation and paw swelling[24]. These data indicate that optimal treatment for rheumatoid arthritis might require combined JNK1 and 2 inhibition. The JNK inhibitor SP-600125 also reduces bronchoalveolar accumulation of eosinophils and lymphocytes in animals subjected to repeated allergen exposure, and reduces serum Immunoglobulin E levels, indicating its possible use in the treatment of asthma[25].

Inappropriate activation of T lymphocytes initiates and perpetuates many autoimmune diseases, including asthma, inflammatory bowel disease and multiple sclerosis. Studies of immature T cells (thymocytes) have not demonstrated a role for JNK in early development. However, CD3-mediated apoptosis of $CD4^+$ $CD8^+$ double-positive thymocytes caused by the administration of a monoclonal antibody to CD3 *in vivo* is reduced in *JnK*-null mice[26,27]. This observation indicates that JNK might contribute to negative selection of autoreactive T cells in the thymus, but a direct test of this hypothesis has not yet been carried out. Exposure of $CD4^+$ T cells to antigen causes these cells to differentiate into effector T_H1 or T_H2 cells, which secrete cytokines that control the type of immune response that is generated. T_H1 cells promote cell-mediated immunity against intracellular microbial pathogens by expressing interferon-γ (IFN-γ), IL-2 and lymphotoxin. By contrast, T_H2 cells express cytokines (IL-4, IL-5, IL-9, IL-10 and IL-13) that promote humoral immunity against parasites and extracellular pathogens. Studies of compound mutations of *JnK1* and *JnK2* demonstrate that JNK is not required for $CD4^+$ T cell activation, but that JNK is required for differentiation to effector cells[28]. So, *JnK1*-null $CD4^+$ T cells selectively differentiate into T_H2 effector cells[29]. Similarly, *JnK2*-null $CD4^+$ T cells produce less IFN-γ during differentiation and, as a result, express low levels of the β2 subunit of the IL-12 receptor and fail to differentiate into the T_H1 subtype[30].

$CD8^+$ T cells can differentiate into cytotoxic T cells that help defend the host during cell-mediated immune responses by secretion of IFN-γ, TNF-α, perforin and granzyme. Interestingly, JNK1 and JNK2 seem to have different roles in this response[31,32]. Studies of *JnK1*-null mice demonstrate that antigen-driven $CD8^+$ T-cell expansion is severely reduced *in vivo* and *in vitro*. This defect is associated with reduced AP-1 transcription activity and reduced cytokine expression (IL-2 and IFN-γ) and is caused, at least in part, by failure of the $CD8^+$ cells to express CD25, the α chain of the IL-2 receptor. By contrast, *JnK2*-null $CD8^+$ T cells express greatly increased amounts of IL-2 and IFN-γ. Together, these data indicate that JNK1 and JNK2 have positive and negative roles, respectively, in the $CD8^+$ T-cell immune response. These different roles of JNK1 and JNK2 indicate that the effect of pharmacological inhibition of JNK in $CD8^+$ T cells could be complex. Nevertheless, studies using the small-molecule JNK inhibitor SP-600125 demonstrate that this drug mimics JNK1 deficiency by inhibiting antigen-driven $CD8^+$ T-cell expansion and severely reduces the expression of IL-2

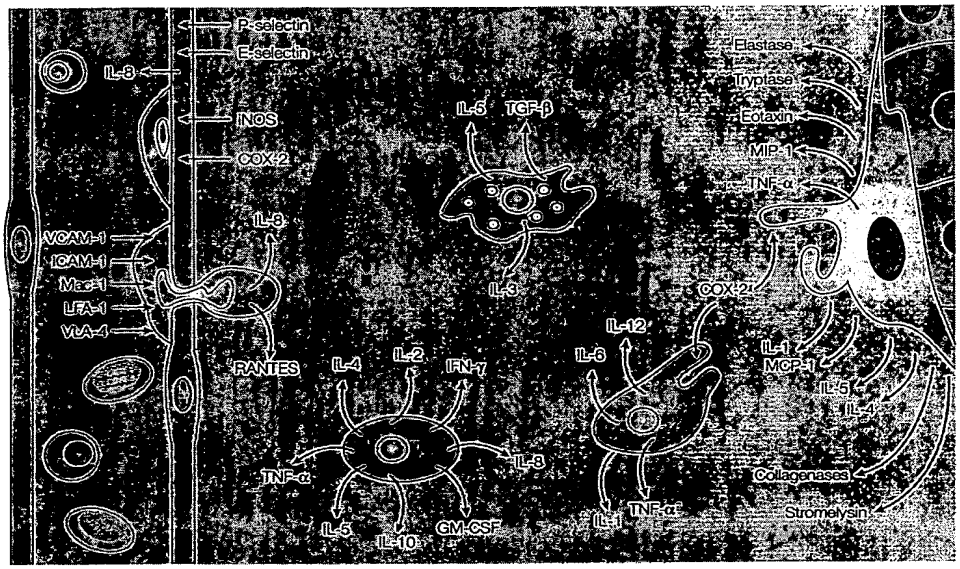

Figure 4 | **Inflammatory gene expression and JNKs.** Many autoimmune diseases originate from an imbalance in normal immune responses to tissue injury, infection or immune surveillance. In response to these stimuli, vascular endothelium surrounding tissues is activated, resulting in increased expression of endothelial cell adhesion molecules such as P- and E-selectin, intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) and chemotactic cytokines such as interleukin-8 (IL-8) or RANTES. These molecules promote the margination, activation and extravasation of blood leukocytes. These leukocytes, including T lymphocytes, macrophages and neutrophils, are activated during this process and express distinct sets of secreted products that contribute to the resolution of tissue trauma or infection. Local cells, including resident mast cells or tissue macrophages, and epithelial and fibroblast-like cells, are also activated and express discrete sets of products. Under normal homeostatic conditions, an inflammatory response will resolve itself. However, in autoimmune disease, the inflammatory response is not resolved, with chronic inflammation leading to significant tissue destruction and remodelling. Many of the gene products expressed by the activated cells involved in the inflammatory response are regulated by the transcription factor activator protein-1 (AP-1), and the c-Jun NH_2-terminal kinase (JNK) pathway. COX-2, cyclooxygenase-2; GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN-γ, interferon-γ; iNOS, inducible nitric oxide synthase; LFA-1, lymphocyte function associated protein-1; Mac-1, macrophage antigen α-polypeptide; MCP-1, membrane cofactor protein-1; MIP-1, major intrinsic protein-1; TGF-β, transforming growth factor-β; TNF-α, tumour-necrosis factor-α; VLA-4, integrin α4.

and IFN-γ[32]. This observation is consistent with the results of biochemical measurements of JNK activity that indicate the presence of high levels of JNK1 activity and low levels of JNK2 activity in activated $CD8^+$ T cells.

The JNK pathway is activated in T cells by antigen stimulation and CD28 receptor co-stimulation, and regulates production of the growth factor IL-2 and cellular proliferation[33]. T cells activated by antigen receptor stimulation in the absence of accessory cell-derived co-stimulatory signals lose the capacity to synthesize IL-2, a state called clonal anergy. This is an important process by which autoreactive T-cell populations are eliminated from the peripheral circulation. Of note is that anergic T cells fail to activate the JNK pathway in response to CD3- and CD28-receptor co-stimulation, even though expression of the JNK enzymes is unchanged[34].

In summary, JNKs seems to play multiple roles in T-cell immune responses[35]. JNK inhibition does not block $CD4^+$ T-cell activation, but does selectively inhibit T_H1-mediated immune responses. By contrast, JNK inhibition does inhibit $CD8^+$ T-cell activation. These data indicate that JNK is a potential therapeutic target that might allow the selective modulation of effector T cell function in diseases such as rheumatoid arthritis, asthma and chronic transplant rejection.

NEUROFIBRILLARY TANGLES Intracellular aggregates of paired helical filaments composed primarily of hyper-phosphorylated Tau proteins. Tau is a microtubule-associated protein found within neurons and normally restricted to axons. Hyper-phosphorylated Tau forms tangled masses that consume the neuronal cell body, presumably leading to neuronal dysfunction and ultimately cell death.

Neurodegenerative diseases

Neurodegenerative diseases, including Alzheimer's, Parkinson's and Huntington's diseases and stroke, share synaptic loss, neuronal atrophy and death as common pathological hallmarks. During the past decade, pharmaceutical research on these diseases has shifted focus from symptomatic benefit to developing novel disease-modifying agents. A key driver of this focus is the enhancement in fundamental knowledge of the mechanisms governing neuronal survival and death. JNK plays an integral role in neuronal death and this pathway might be operative in various central nervous system (CNS) disease states (FIG. 5).

JNKs 1 and 2 exhibit a broad tissue distribution, whereas JNK3 is predominantly localized to brain and testes. In the human CNS, the major JNK isoforms expressed are JNK3α1 and JNK1α1 (REF. 36), with JNK3 preferentially localized to pyramidal neurons in the CA1

and CA2 regions of the hippocampus and in specific subregions of the neocortex.

Knockout mice lacking either *JnK1*, *JnK2* or *JnK3* develop normally[36,37]. However, mice lacking both JNKs 1 and 2 die prematurely and exhibit brain abnormalities that are attributable to a dysregulation of apoptosis. So, JNK1 and JNK2 might be redundant in function for embryonic brain development. Although, disruption of *JnK3* in developing mice is of no apparent consequence, a pathological role for JNK3 downstream of stress-inducing stimuli is evident. In cell culture, increased expression of c-Jun, c-Jun phosphorylation and/or JNK activity correlates with neuronal apoptosis induced by a variety of stimuli[38,39]. JNK3 deficiency blocked c-Jun phosphorylation, c-Jun induction and sympathetic neuron death following trophic factor withdrawal[40].

Alzheimer's disease is characterized by progressive memory loss and deterioration of cognitive function. Pathologically, the hallmarks of Alzheimer's disease include a prevalence of amyloid deposits, NEUROFIBRILLARY TANGLES (NFTs) and neuronal synapse and cell loss, predominantly in the cortex and hippocampus. Genetic analyses of familial Alzheimer's disease cases provided links to mutations in the amyloid precursor protein (APP) and the presenilin genes *PSEN1* and *PSEN2*, which might therefore have a role in Alzheimer's disease pathogenesis. Extending these findings, animal models revealed that the identified mutations in the APP and the *PSEN1* and *PSEN2* genes affect the generation and deposition of β-amyloid fragments[41]. Direct toxicity of β-amyloid is postulated to contribute to the neuronal dysfunction and loss observed in Alzheimer's disease. *In vitro* exposure of neurons to β-amyloid fragments results in neurite atrophy and cell death with morphological and biochemical characteristics consistent with an apoptotic process. β-Amyloid-induced cell death is attenuated in cortical neurons from *JnK3*-null mice, and JNK3 mediates this cell death through the activation of c-Jun and the enhanced expression of Fas ligand[42].

Post-mortem brain sections from Alzheimer's disease patients revealed an altered distribution and activation of JNKs. JNKs 1, 2 and 3 distributed to different subcellular structures specific to the Alzheimer's disease brain[43,44], thereby indicating either a causal role in, or a response to, the pathology. JNK phosphorylates Tau *in vitro* at sites identified in paired helical filament Tau, the major constituent of NFTs[45]. Phospho-JNK staining was also localized in transgenic mice overexpressing a mutant form of PSEN1 to neurons surrounding amyloid plaques, as well as to neurons that contained intracellular accumulations of β-amyloid[46].

A direct role for the JNK pathway in functional regulation and metabolism of APP has also been postulated. APP is a substrate for JNK3 (REF. 47). The JNK phosphorylation site of APP is Thr668, a site that is also phosphorylated by MAPK3 (also known as extracellular-regulated kinase-1 (ERK1)), glycogen synthase kinase 3β (GSK3β), and CDK5/p35 (although each of these kinases phosphorylates APP to a lesser extent than JNK). Clearly, signalling through the JNK pathway is relevant to multiple physiological and pathological events that might be operative in Alzheimer's disease.

Parkinson's disease is characterized by behavioural impairments resulting from the relatively selective death of dopaminergic neurons. Similarly to Alzheimer's disease, the neuronal loss in Parkinson's disease is progressive and occurs over an extended timeframe. Several studies examining Parkinson's disease autopsy brains revealed that apoptosis could be the underlying mode of death of the vulnerable neurons[48]. So far, there is no direct evidence of JNK activation in Parkinson's disease autopsy brains, so its involvement in the pathological process is inferred from the results of cell culture and animal model studies.

The best characterized and most relevant animal models of Parkinson's disease use the selective nigrostriatal dopaminergic neurotoxin 1-methyl-4-phenyl-tetrahydropyridine (MPTP). MPTP administration to experimental animals produces a pattern of neurodegeneration and a neuropathology that is nearly identical to that seen in the brains of post-mortem human Parkinson's disease specimens. Recently, adenoviral gene transfer of the JNK-binding domain of JIP-1, which acts as an inhibitor of JNK function, into the striatum of mice inhibited MPTP-mediated activation of JNK, c-Jun and caspase in the substantia nigra. This treatment also blocked neuronal death in the substantia nigra and the loss of catecholamines in dopaminergic terminals[49]. This agent attenuated behavioural impairment as measured by amphetamine-induced locomotor increases.

Ischaemic injury to the CNS can lead to neuronal injury and death through a number of mechanisms. Several factors have been defined that mediate cell death after ischaemia, including excitotoxicity, elevated intracellular calcium levels, inflammatory processes mediated by cytokines and loss of survival factors[50]. In a model of transient focal ischaemia, elevated levels of phospho-c-Jun co-localized with TUNEL-labelled neurons in the cortex, which indicates the activation of upstream JNKs[51]. The co-localization with TUNEL-labelled neurons indicates a causative role for JNK activation and apoptosis.

Mice deficient in JNK3 or expressing mutations in the phosphorylation site of c-Jun are resistant to the hippocampal neurotoxic events associated with administration of the glutamate receptor agonist kainic acid. Mice that are deficient in JNK1 or JNK2 are not resistant to either kainic acid-mediated seizures or neuronal death[37]. These data indicate that the different JNK isoforms regulate differential responses to neuronal insults; in particular, the JNK3 isoform is involved in glutamate excitotoxicity, an important component in ischaemic death, and is expressed in a brain region that is vulnerable to global ischaemic conditions.

Metabolic disease

Obesity and type 2 diabetes are the most prevalent and serious of the metabolic diseases[52]. Insulin resistance is closely associated with these syndromes, and is commonly evident in hypertension, and following infection and injury. In these settings, β-cells within the pancreas

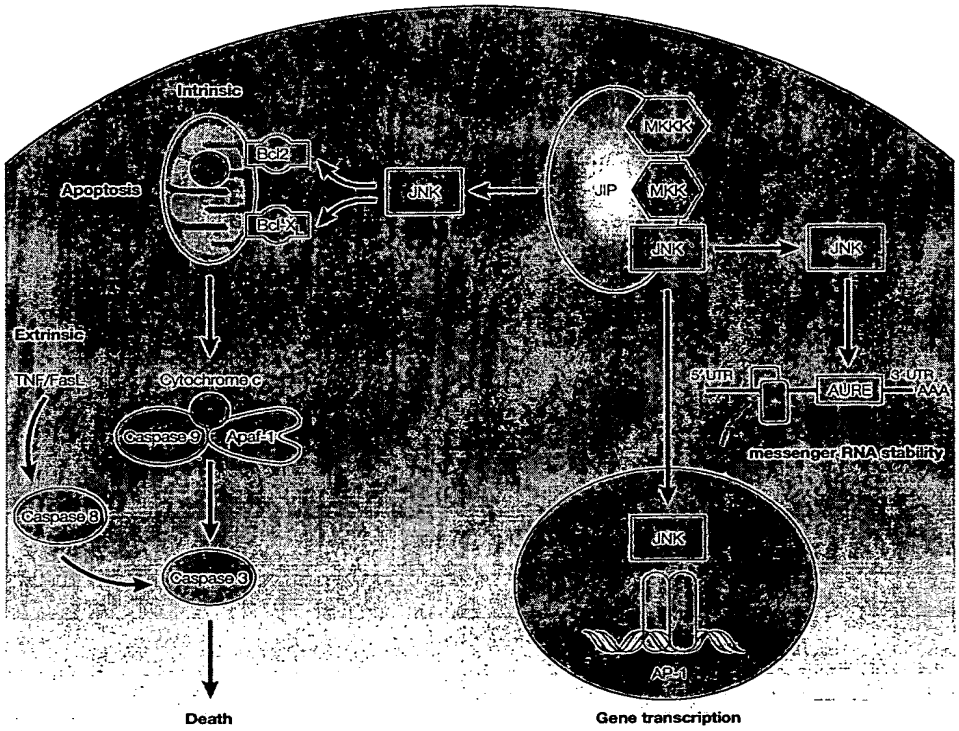

Figure 5 | **Biological functions of JNK.** C-Jun N-terminal kinases (JNKs) regulate cell survival and apoptosis by distinct mechanisms. JNKs play a key role in regulating the transcription and translation of cellular genes involved in the stress response. Activated JNKs interact with activator protein-1 (AP-1) and other transcription factors to modulate transcription of a number of genes. JNKs can also modulate the half-life of a set of genes that contain AU-rich elements (AURE) in their 3' untranslated regions (UTR), an element associated with rapid turnover and short half-life. Activation of JNKs results in a repression of turnover via these elements, thereby enhancing translation of these messenger RNAs. Activated JNKs can also promote cellular apoptosis by activating an intrinsic pathway whereby Bcl2 (B-cell lymphomas 2) and BCL-xL promote release of pro-apoptotic molecules including cytochrome c from mitochondria. This leads to the activation of caspases and cell death. JIP, JNK-interacting protein; MKK, mitogen-activated protein (MAP) kinase kinase; MKKK, MAP kinase kinase; TNF, tumour-necrosis factor.

fail to secrete sufficient insulin to compensate for peripheral insulin resistance. Insulin resistance and compensatory hyper-insulinaemia dysregulate many physiological processes that contribute to life-threatening metabolic, vascular and cardiac diseases[53]. Although new drugs are emerging to improve insulin sensitivity, the molecular mechanisms of insulin resistance have been the subject of intensive research. The idea that inflammation causes insulin resistance has been held for some time and is consistent with the concept that anti-inflammatory drugs, such as high dose aspirin, promote insulin sensitivity. The physiological response to infection, physical or thermal injury, or obesity invariably involves the production of pro-inflammatory cytokines, such as TNF-α, that activate various serine kinases. Considerable evidence indicates that serine phosphorylation of the insulin receptor or the insulin receptor substrate (IRS) proteins might inhibit insulin signalling and promote insulin resistance.

During obesity, adipocytes produce TNF-α, which promotes insulin resistance and stimulates serine phosphorylation of IRS1, whereas disruption of TNF receptor-1 partially restores insulin signalling and glucose tolerance in obese mice[54]. Insulin signalling complexes are assembled by insulin-stimulated tyrosine phosphorylation of scaffold proteins, including the IRS proteins, Src homology 2 domain-containing transforming protein, adaptor protein with pleckstrin homology and src homology 2 domains, GABA (γ-aminobutyric acid) receptors 1/2, Cas-Br-M (murine) ecotropic retroviral transforming sequence. Though the role of each of these components is of interest, transgenic mice studies revealed the importance of Irs1 and Irs2 for somatic growth and carbohydrate metabolism[55,56]. More than 100 potential serine phosphorylation sites exist in Irs1, and many protein kinases phosphorylate Irs1 *in vitro*, including JNK. The Irs proteins contain a binding site for the docking

domain of JNK. Activation of JNK by pro-inflammatory cytokines inhibits insulin signalling in mouse embryonic fibroblasts, 3T3-L1 and 32Dir cells through phosphorylation of Ser307 of Irs1. Insulin-stimulated Irs1 Ser307 phosphorylation was inhibited by almost 80% in cells lacking JNK1 or JNK2, or in cells expressing a mutant Irs1 protein lacking the JNK-binding domain[57]. Insulin activates JNK activity in other cell types, including L6 monocytes, rat adipocytes and Rat-1 fibroblasts, indicating that activated JNK could be an important negative feedback regulator of insulin signalling[58].

Striking evidence for a role of JNK in insulin resistance and obesity came from the finding that mice fed on a high-fat diet, and *ob/ob* mice that are genetically prone to obesity, exhibit spontaneously high JNK1 activity in liver, skeletal muscle and fat. Mice lacking JNK1 show decreased adiposity, significantly improved insulin sensitivity and enhanced insulin receptor signalling in the high-fat and *ob/ob* models[59]. As a mediator of obesity and insulin resistance, as well as many other cellular processes including apoptosis and neural differentiation, JNK is a potential therapeutic target for obesity and type 2 diabetes.

Cancer

The causal routes by which signal transduction pathways contribute to cellular transformation and tumorigenesis are well established. A substantial body of evidence indicates that JNK activation and c-Jun phosphorylation are required for transformation induced by *RAS*, an oncogene that is mutationally activated in almost 30% of human cancers[60]. Ras induces phosphorylation of c-Jun on the same serine residues phosphorylated by JNK[4,61], and acts cooperatively with c-Jun to enhance cellular transformation[62]. Fibroblasts lacking c-Jun cannot be transformed by Ras, which indicates a requirement for c-Jun in this process[63]. Moreover, recent studies have demonstrated the requirement of c-Jun for the development of chemically induced liver tumours in a model of hepatocellular carcinoma[64]. One of the functions of c-Jun that might contribute to tumour development is the transcriptional repression of the gene that encodes the p53 tumour suppressor[65]. Taken together, these data strongly support a role for c-Jun in cellular transformation.

JNK also seems to play a significant role in tumour development. Several tumour cell lines have been reported to possess constitutively active JNK[66]. The transforming potential of several oncogenes is reduced after the introduction of antisense JNK oligonucleotides or dominant-negative versions of proteins belonging to the JNK pathway[67,68]. A series of transfection studies demonstrated that the sites of c-Jun phosphorylation by JNK are required for efficient co-transformation activity with activated Ras[61]. Moreover, fibroblasts from mice harbouring a mutated *c-Jun* allele that lacks the JNK phosphorylation sites (JunAA) are resistant to transformation induced by activated Ras and Fos[69]. c-Fos-induced osteosarcomas and skin tumours induced by chronic activation of the Ras pathway are reduced in JunAA mice. Collectively, these data indicate that JNK activity is necessary for efficient transformation and tumorigenesis by oncogenes such as *Ras*.

However, in a recent study, fibroblasts isolated from mice that lack expression of JNK due to compound mutations of the *JnK* genes were efficiently transformed by Ras, and actually formed increased numbers of tumour nodules and size of individual tumours in mice injected with these cells[70]. This enhanced tumour formation seemed to be due to the absence of JNK-stimulated apoptosis in Ras-induced *JnK*-null tumours. An important aspect of tumour development is the suppression of apoptosis, and human tumours seem to utilize several different mechanisms to evade cell suicide, including enhanced expression of B-cell leukaemia/lymphoma 2 (Bcl2), p53 and apoptotic protease activating factor-1. So, a normal function of JNK might be to suppress tumour formation by activating apoptosis. How might this be mediated? Examination of *Jnk*-null primary fibroblasts has shown that JNK is necessary for stress-induced mitochondrial release of proapoptotic molecules, including cytochrome *c* (see FIG. 3)[9]. Activated JNK is sufficient to cause caspase-independent release of cytochrome *c* and subsequent apoptosis. Studies of fibroblasts have revealed a requirement for both Bax and Bak — pro-apoptotic members of the Bcl2 family — in JNK-mediated cytochrome *c* release and apoptosis[71]. Together, these data indicate that JNK activates apoptosis by interactions with the Bcl2 family of proteins. Therefore, it is possible that JNK might promote or suppress tumour development in different settings.

It is probable that JNK1 and JNK2 are not tumour suppressors, because they are ubiquitously expressed and exhibit a strong degree of functional redundancy, and the likelihood of mutational loss of both *JNK* genes is extremely low. However, JNK3 expression is largely restricted to the brain and has functions that are not compensated for by JNK1 or JNK2. Indeed, since mutations in *JNK3* were identified in ten out of nineteen human brain tumours examined[72], *JNK3* can be considered a candidate tumour suppressor gene. Similarly, the upstream activators of JNK (MAP2K4 and MAP2K7) serve nonredundant roles and so could also be tumour suppressor genes[73,74]. It is interesting in this regard that mutations in *MAP2K4* have been identified in human cancers of the pancreas, lung, breast, colon and prostate[75,76]. Interestingly, previous studies have identified *MAP2K4* as a metastasis suppressor gene. Loss-of-function mutations in *MAP2K4* are associated with aggressive growth and metastasis of prostate and ovarian cancers[77,78]. These data are consistent with the recently published study of *JNK*-null fibroblasts that indicate a role for JNK in metastasis suppression[70]. However, a direct test of this hypothesis in a reliable animal model of metastasis will be required to confirm whether JNK plays a role in metastasis suppression, or in tumour surveillance.

Although JNK seems to act as a tumour suppressor in fibroblast transformation caused by oncogenic Ras, JNK can contribute to proliferation or survival of other tumour cell types. JNK can potentiate B-cell lymphoma

caused by breakpoint cluster region-Abelson murine leukaemia oncogene, because JNK is required for tumour cell survival[79]. Studies of various cancer cell lines have revealed high levels of JNK activity[66], and inhibition of JNK using antisense approaches can reduce oncogenic transformation in some tumour cell lines[67,68].

Together, these considerations indicate that JNK could play more than one role in tumour development, and that in certain cases this role might be to promote or inhibit tumour development. Gaining a deeper understanding of the genetic and mechanistic basis for these different roles of JNK in tumours is essential for determining the true potential of JNK inhibitors as anticancer therapeutics.

JNK drug discovery

During the past decade, a combination of high-throughput screening, kinase-specific libraries and structure-based drug design has facilitated the discovery of selective kinase inhibitors. Screening of natural products of different origin, and collections of available compounds, has led to the identification of compounds that served as templates for medicinal chemistry efforts to design selective inhibitors for a range of kinase targets[80]. This information has facilitated the design, synthesis and screening of libraries of compounds that have structural features of inhibitors that interact at the kinase ATP site[81]. Determination of the X-ray structure of the members of the MAPK family, ERK[82], p38 (REF. 83) and JNK3 (REF. 84) has revealed approaches for the design of potent, yet selective, inhibitors of the JNKs. These efforts have led to the patenting of a series of JNK inhibitors, as described below.

Signal Pharmaceuticals (now Celgene) reported the discovery of a series of pyrazoloanthrone derivatives of compound 1 (SP-600125) as inhibitors of the JNK pathway for the treatment of autoimmune, anti-inflammatory and neurodegenerative diseases[23,85]. Compound 1 (FIG. 6) showed IC_{50} values of 110 nM for JNK1 and JNK2, and 150 nM for JNK3. Compound 1 was also evaluated for selectivity against a number of kinases and showed greater than 30 μM inhibitory activity against p38-2, ERK1, MAP-KKK1, IKK1, inhibitor of κ kinase-β (IKK2), protein kinase A, protein kinase C and epidermal growth factor receptor. Recently, SP-600125 was tested under different conditions against a broader range of kinases, in which it inhibited several other kinases with a similar or greater potency than the JNKs[86]. The true selectivity of this compound has yet to be resolved, but it does seem to be a valuable tool for assessing the role of JNK in various disease models. In cellular assays, SP-600125 inhibited TNF-α production in monocytes and IL-2 production in Jurkat cells with an IC_{50} of approximately 5 μM. In rats, compound 3, administered 15 minutes before lipopolysaccharide, blocked TNF-α production when dosed intravenously and orally. SP-600125 inhibited leukocyte recruitment in a rat model of allergic airway inflammation at 30 mg per kg subcutaneously[25], and blocked JNK activation, MMP3 expression, and joint destruction in a rat adjuvant arthritis model[21]. In the rat, SP-600125 also blocked kainic acid-induced seizure activity by approximately 30%.

A second series of JNK-selective inhibitors (compound 2) were also recently disclosed from Celgene[87]. Celgene initiated a single, escalating-dose Phase I safety trial in normal healthy volunteers late in 2002 (REF. 88). The identity of the JNK inhibitor compound under investigation is unknown at this time.

A series of pyrimidinyl-substituted benzazole-acetonitriles (compound 3; FIG. 6) designed by Serono were disclosed as inhibitors of JNK2 and JNK3 for the treatment of autoimmune and neuronal diseases[89]. The benzazoles are more potent inhibitors of JNK3 than JNK2, with several compounds inhibiting JNK3 in the 30–70 nM range. Serono also disclosed a large series of sulfonyl amino acid[90], sulfonamide[91] and sulfonyl hydrazides[92] (compound 4) as inhibitors of JNK2 and JNK3. The inhibitors were reported to promote survival of sympathetic neurons in culture and to protect against cell death during stroke following global ischaemia in gerbils. It is not known whether these inhibitors are competitive with the ATP site, with the substrate site, or are producing inhibition in a noncompetitive manner.

Vertex reported a series of 3-oximido-oxindole analogues (compound 5) for the treatment of stroke and neurodegenerative diseases[93]. A number of compounds containing benzo-1,3-dioxolane groups inhibited JNK3 with IC_{50} values less than 100 nM. Vertex also disclosed a series of 4-substituted isoxazole analogues (compound 6) containing a 2-anilinopyridine or 2-anilinopyrimidines as JNK3 inhibitors[94]. A variety of substituents were tolerated on the aromatic rings, resulting in potent JNK3 inhibitors.

Researchers at Takeda disclosed the preparation of azoles as JNK inhibitors (compound 7)[95]. These compounds showed *in vitro* IC_{50} values of 30–210 nM against JNK1. In an *in vitro* assay using THP-1 cells, compounds of this class inhibited TNF-α production with IC_{50} values of 2–100 nM.

Hoffman-LaRoche reported a series of 4-aryl- (compound 10) and 4-alkynyl- (compounds 11–13) isoindolones (compound 8) as inhibitors of the JNKs[96,97]. Several alkynyl analogues inhibited JNK/SAPK with IC_{50} values less than 150 nM.

Hoffman-LaRoche also disclosed a series of 4,5-pyridazinoxindole JNK inhibitors (compound 9) for the treatment of neurodegenerative and inflammatory diseases[98]. A variety of substitutions were tolerated on the pyridazine moiety. A derivative in which R1 and R2 are simple alkyl or fused cycloalkyl groups, such as cyclohexyl 17, exhibited IC_{50} values below 100 nM for JNKs.

Recently, researchers at Aventis claimed new substituted indolizine derivatives (exemplified by compound 10) as JNK inhibitors useful for the treatment of cancer, asthma and arthritic diseases[99]. No pharmacological data for these compounds has been reported.

A series of 1H-indazole derivatives of compound 11 from Eisai were reported as JNK inhibitors with

IC_{50} values of about 50 nM against JNK3, and as being useful for the treatment and prevention of Alzheimer's and Parkinson's diseases[100]. Additionally, imidazole derivatives of compound 11 were claimed to be potent JNK3 inhibitors with IC_{50} values of 6 nM against JNK3 (REF. 101).

Researchers at Merck claimed the use of 4-(4-pyrimidinyl)-5-phenylimidazole derivatives as JNK inhibitors[102]. Compound 12 was reported to inhibit JNK3α1 *in vitro* with an IC_{50} of 1 nM. These compounds were claimed to have utility as apoptosis inhibitors for the treatment and prevention of stroke, Parkinson's disease,

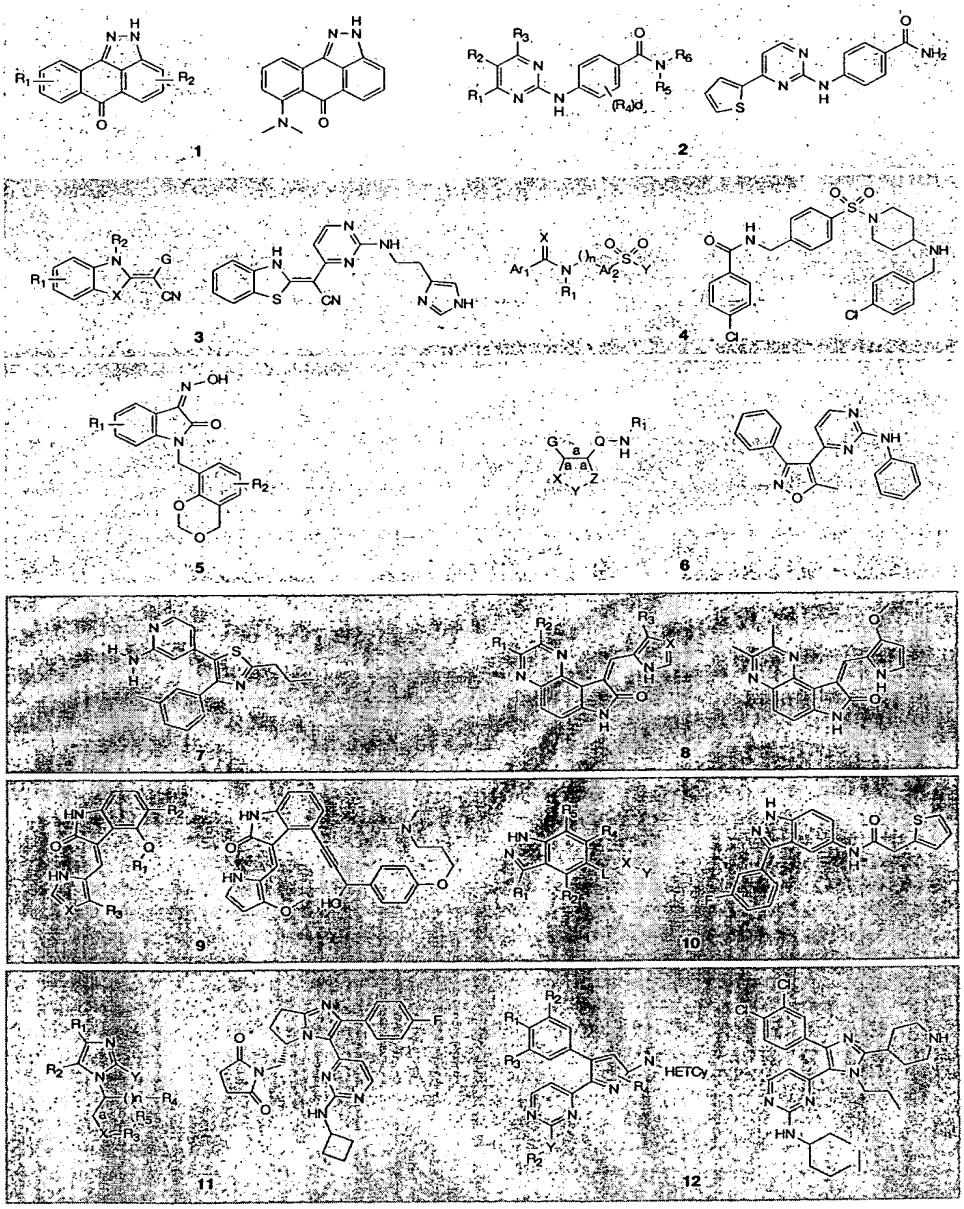

Figure 6 | **Inhibitors of JNKs reported in the patent literature.** In most cases, the generic core structure is represented on the left, with specific examples cited on the right. JNK, c-Jun NH_2-terminal kinase.

Alzheimer's disease, amylotrophic lateral sclerosis, multiple sclerosis, spinal cord injury, head trauma and seizures. No pharmacological data have been disclosed.

Conclusions

Data continues to emerge implicating the JNK pathway in a number of physiological and pathological functions that are probably operative in a range of human diseases. The shear breadth of the diseases in which JNK inhibitors could show benefit has attracted many pharmaceutical companies seeking blockbuster opportunities and maximal return on their research investment. Our understanding of the organization and function of all levels within the JNK signalling cascade continues to evolve. Because of the complex cross-talk within this signalling cascade, as well as its cell-type- and response-specific modulation, it is difficult to predict potential adverse events that might arise from pathway inhibition. The fact that compounds that inhibit the JNK pathway are progressing in clinical trials bears hope that sufficient safety and risk–benefit margins will be observed. In the coming years, the utility of targeting this pathway for therapeutic benefit will probably be determined.

1. Kyriakis, J. M. & Avruch, J. pp54 microtubule-associated protein 2 kinase. A novel serine/threonine protein kinase regulated by phosphorylation and stimulated by poly-L-lysine. *J. Biol. Chem.* **265**, 17355–17363 (1990).
2. Hibi, M., Lin, A., Smeal, T., Minden, A. & Karin, M. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev.* **7**, 2135–2148 (1993).
3. Adler, V., Polotskaya, A., Wagner, F. & Kraft, A. S. Affinity-purified c-Jun amino-terminal protein kinase requires serine/threonine phosphorylation for activity. *J. Biol. Chem.* **267**, 17001–17005 (1992).
4. Derijard, B. *et al.* JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* **76**, 1025–1037 (1994).
5. Kyriakis, J. M. *et al.* The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* **369**, 156–160 (1994).
6. Davis, R. J. Signal transduction by the JNK group of MAP kinases. *Cell* **103**, 239–252 (2000).
A concise overview of the components of the three mitogen-activated protein kinase signalling pathways and their therapeutic potential.
7. Gupta, S. *et al.* Selective interaction of JNK protein kinase isoforms with transcription factors. *EMBO J.* **15**, 2760–2770 (1996).
8. Ventura, J. J., Kennedy, N. J., Lamb, J. A., Flavell, R. A. & Davis, R. J. c-Jun NH_2-terminal kinase is essential for the regulation of AP-1 by tumor necrosis factor. *Mol. Cell Biol.* **23**, 2871–2882 (2003).
9. Tournier, C. *et al.* Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. *Science* **288**, 870–874 (2000).
10. Lawler, S., Fleming, Y., Goedert, M. & Cohen, P. Synergistic activation of SAPK1/JNK1 by two MAP kinase kinases *in vitro*. *Curr. Biol.* **8**, 1387–1390 (1998).
11. Tournier, C. *et al.* MKK7 is an essential component of the JNK signal transduction pathway activated by proinflammatory cytokines. *Genes Dev.* **15**, 1419–1426 (2001).
12. Ensler, H. & Davis, R. J. Regulation of MAP kinases by docking domains. *Biol. Cell* **93**, 5–14 (2001).
13. Morrison, D. & Davis, R. J. MAP kinase scaffold proteins in mammals. *Annu. Rev. Dev. Cell Biol.* (in the press).
14. Harper, S. J. & LoGrasso, P. Inhibitors of the JNK signaling pathway. *Drugs of the Future* **26**, 957–973 (2001).
An excellent review of progress in the discovery of inhibitors of JNK signalling, including recent patent activity.
15. Maroney, A. C. *et al.* CEP-1347 (KT7515), a synthetic inhibitor of the mixed lineage kinase family. *J. Biol. Chem.* **276**, 25302–25308 (2001).
A detailed description of an inhibitor of JNK signalling that functions upstream of the JNKs.
16. Manning, A. M. & Mercurio, F. Transcription inhibitors in inflammation. *Exp. Opin. Invest. Drugs* **6**, 555–567 (1997).
17. Swantek, J. L., Cobb, M. H. & Geppert, T. D. Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) is required for lipopolysaccharide stimulation of tumor necrosis factor-α (TNF-α) translation: glucocorticoids inhibit TNF-α translation by blocking JNK/SAPK. *Mol. Cell. Biol.* **17**, 6274–6282 (1997).
18. Ishizuka, T. *et al.* Mast cell tumor necrosis factor α production is regulated by MEK kinases. *Proc. Natl Acad. Sci. USA* **94**, 6358–6363 (1997).
19. Gum, R., Wang, H., Lengyel, E., Juarez, J. & Boyd, D. Regulation of 92 kDa type IV collagenase expression by the jun aminoterminal kinase- and the extracellular signal-regulated kinase-dependent signaling cascades. *Oncogene* **14**, 1481–1493 (1997).
20. Han, Z. *et al.* Jun-N-terminal kinase in rheumatoid arthritis. *J. Pharm. Exp. Therap.* **291**, 124–130 (1999).
21. Han, Z. *et al.* c-Jun N-terminal kinase is required for metalloproteinase (MMP) expression in synoviocytes and regulates bone destruction in adjuvant arthritis. *J. Clin. Invest.* **108**, 73–81 (2001).
22. Clancy, R. *et al.* Activation of stress-activated protein kinase in osteoarthritis cartilage: evidence for nitric oxide dependence. *Osteoarthritis Cartilage* **9**, 294–299 (2002).
23. Bennett, B. L. *et al.* SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. *Proc. Natl Acad. Sci. USA* **98**, 13681–13686 (2001).
The first detailed description of the pharmacologic profile of a selective JNK inhibitor.
24. Han, Z., Chang, L., Yamanishi, Y., Karin, M. & Firestein, G. S. Joint damage and inflammation in c-Jun N-terminal kinase 2 knockout mice with passive murine collagen-induced arthritis. *Arthritis Rheum.* **46**, 818–823 (2002).
25. Eynott, P. R., Adcock, I. M. & Chung, P. The effects of selective c-Jun N-terminal kinase inhibition in a sensitized Brown Norway rat model of allergic asthma. *Am. J. Respir. Crit. Care Med.* **49**, S102 (2001).
26. Sabapathy, K. *et al.* JNK2 is required for efficient T-cell activation and apoptosis but not for normal lymphocyte development. *Curr. Biol.* **9**, 116–125 (1999).
27. Rincon, M. *et al.* The JNK pathway regulates the *in vivo* deletion of immature $CD4^+CD8^+$ thymocytes. *J. Exp. Med.* **188**, 1817–1830 (1998).
28. Dong, C. *et al.* JNK is required for effector T-cell function but not for T-cell activation. *Nature* **405**, 91–94 (2000).
29. Dong, C. *et al.* Defective T cell differentiation in the absence of Jnk1. *Science* **282**, 2092–2095 (1998).
30. Yang, D. D. *et al.* Differentiation of $CD4^+$ T cells to Th1 cells requires MAP kinase JNK2. *Immunity* **9**, 575–585 (1998).
31. Arbour, N. *et al.* c-Jun NH_2-terminal kinase (JNK)1 and JNK2 signaling pathways have divergent roles in $CD8^+$ T cell-mediated antiviral immunity. *J. Exp. Med.* **195**, 801–810 (2002).
32. Conze, D. *et al.* c-Jun NH_2-terminal kinase (JNK)1 and JNK2 have distinct roles in $CD8^+$ T cell activation. *J. Exp. Med.* **195**, 811–823 (2002).
33. Su, B. *et al.* JNK is involved in signal integration during costimulation of T lymphocytes. *Cell* **77**, 727–736 (1994).
34. Li, W., Whaley, C. D., Mondino, A. & Mueller, D. L. Blocked signal transduction to the ERK and JNK protein kinases in anergic $CD4^+$ T cells. *Science* **271**, 1272–1276 (1996).
35. Rincon, M., Flavell, R. A. & Davis, R. J. Signal transduction by MAP kinases in T lymphocytes. *Oncogene* **20**, 2490–2497 (2001)
36. Kuan, C. Y. *et al.* The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. *Neuron* **22**, 667–676 (1999).
37. Yang, D. D. *et al.* Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the *Jnk3* gene. *Nature* **389**, 865–870 (1997).
38. Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J. & Greenberg, M. E. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* **270**, 1326–1331 (1995).
39. Le-Niculescu, H. *et al.* Withdrawal of survival factors results in activation of the JNK pathway in neuronal cells leading to Fas ligand induction and cell death. *Mol. Cell Biol.* **19**, 751–763 (1999).
40. Bruckner, S. R. *et al.* JNK3 contributes to c-Jun activation and apoptosis but not oxidative stress in nerve growth factor-deprived sympathetic neurons. *J. Neurochem.* **78**, 298–303 (2001).
41. Scheuner, D. *et al.* Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and *APP* mutations linked to familial Alzheimer's disease. *Nature Med.* **2**, 864–870 (1996).
42. Morishima, Y. *et al.* β-amyloid induces neuronal apoptosis via a mechanism that involves the c-Jun N-terminal kinase pathway and the induction of Fas ligand. *J. Neurosci.* **21**, 7551–7560 (2001).
43. Zhu, X. *et al.* Activation and redistribution of c-Jun N-terminal kinase/stress activated protein kinase in degenerating neurons in Alzheimer's disease *J. Neurochem.* **76**, 435–441 (2001).
44. Pei, J-J. *et al.* Localization of active forms of c-Jun kinase (JNK) and p38 kinase in Alzheimer's disease brains at different stages of neurofibrillary degeneration. *J. Alzheimer's Dis.* **3**, 41–48 (2001).
45. Reynolds, C. H., Utton, M. A., Gibb, G. M., Yates, A. & Anderton, B. H. Stress-activated protein kinase/c-Jun N-terminal kinase phosphorylates Tau protein. *J. Neurochem.* **68**, 1736–1744 (1997).
46. Shoji, M. *et al.* JNK activation is associated with intracellular β-amyloid accumulation. *Mol. Brain Res.* **85**, 221–233 (2001).
47. Reynolds, C. H., Betts, J. C., Blackstock, W. P., Nebreda, A. R. & Anderton, B. H. Phosphorylation sites on tau identified by nanoelectrospray mass spectrometry: differences *in vitro* between the mitogen-activated protein kinases ERK2, c-Jun N-terminal kinase and p38, and glycogen synthase kinase-3β. *J. Neurochem.* **74**, 1587–1595 (2001).
48. Anglade, P. *et al.* Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. *Histol. Histopathol.* **12**, 25–31 (1997).
49. Xia, X. G. *et al.* Gene transfer of the JNK interacting protein-1 protects dopaminergic neurons in the MPTP model of Parkinson's disease. *Proc. Natl Acad. Sci. USA* **98**, 10433–10438 (2001).
50. Mattson, M. P. Apoptosis in neurodegenerative disorders. *Nature Rev. Mol. Cell Biol.* **1**, 120–129 (2000).
51. Herdegen, T. *et al.* Lasting N-terminal phosphorylation of c-Jun and activation of c-Jun N-terminal kinases after neuronal injury. *J. Neurosci.* **18**, 5124–5135 (1998).
52. Must, A. *et al.* The disease burden associated with overweight and obesity. *JAMA* **282**, 1523–1529 (1999).
53. Facchini, F. S., Hua, N. W., Reaven, G. M. & Stoohs, R. A. Hyperinsulinemia: the missing link among oxidative stress and age-related diseases? *Free Rad. Biol. Med.* **29**, 1302–1306 (2000).
54. Uysal, K. T., Wiesbrock, S. M., Marino, M. W. & Hotamisligil, G. S. Protection from obesity-induced insulin resistance in mice lacking TNF-α function. *Nature* **389**, 610–614 (1997).
55. Withers, D. J. & White, M. F. Insulin action and type 2 diabetes: lessons from knockout mice. *Curr. Opin. Endocrinol. Diab.* **6**, 141–145 (1999).
56. Withers, D. J. *et al.* Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* **391**, 900–904 (1998).
57. Lee, Y. H., Giraud, J., Davis, R. J. & White, M. F. c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. *J. Biol. Chem.* **278**, 2896–2902 (2003).
58. Standaert, M. L. *et al.* Effects of knockout of the protein kinase C β gene on glucose transport and glucose homeostasis. *Endocrinology* **140**, 4470–4477 (1999).
59. Hirosumi, J. *et al.* A central role for JNK in obesity and insulin resistance. *Nature* **420**, 333–337 (2002).

60. Adjei, A. A. Blocking oncogenic Ras signaling for cancer therapy. *J. Natl Cancer Inst.* **93**, 1062–1074 (2001).
A review of different approaches to Ras inhibition, including targeting JNK.
61. Smeal, T., Binetruy, B., Mercola, D. A., Birrer, M. & Karin, M. Oncogenic and transcriptional cooperation with Ha-Ras requires phosphorylation of c-Jun on serines 63 and 73. *Nature* **354**, 494–496 (1991).
62. Schutte, J., Minna, J. D. & Birrer, M. J. Deregulated expression of human c-Jun transforms primary rat embryo cells in cooperation with an activated *c-Ha-ras* gene and transforms rat-1a cells as a single gene. *Proc. Natl Acad. Sci. USA* **86**, 2257–2261 (1989).
63. Johnson, R., Spiegelman, B., Hanahan, D. & Wisdom, R. Cellular transformation and malignancy induced by ras require c-Jun. *Mol. Cell Biol.* **16**, 4504–4511 (1996).
64. Eferl, R. *et al.* Liver tumor development. c-Jun antagonizes the proapoptotic activity of p53. *Cell* **112**, 181–192 (2003).
65. Schreiber, M. *et al.* Control of cell cycle progression by c-Jun is p53 dependent. *Genes Dev.* **13**, 607–619 (1999).
66. Ip, Y. T. & Davis, R. J. Signal transduction by the c-Jun N-terminal kinase (JNK) — from inflammation to development. *Curr. Opin. Cell Biol.* **10**, 205–219 (1998).
67. Potapova, O. *et al.* The Jun kinase/stress-activated protein kinase pathway functions to regulate DNA repair and inhibition of the pathway sensitizes tumor cells to cisplatin. *J. Biol. Chem.* **272**, 14041–14044 (1997).
68. Potapova, O. *et al.* c-Jun N-terminal kinase is essential for growth of human T98G glioblastoma cells. *J. Biol. Chem.* **275**, 24767–24775 (2000).
69. Behrens, A., Jochum, W., Sibilia, M. & Wagner, E. F. Oncogenic transformation by ras and fos is mediated by c-Jun N-terminal phosphorylation. *Oncogene* **9**, 2657–2663 (2000).
70. Kennedy, N. J. *et al.* Suppression of Ras-stimulated transformation by the JNK signal transduction pathway. *Genes Dev.* **17**, 629–637 (2003).
71. Lei, K. *et al.* The Bax subfamily of Bcl2-related proteins is essential for apoptotic signal transduction by c-Jun NH_2-terminal kinase. *Mol. Cell Biol.* **22**, 4929–4942 (2002).
72. Yoshida, S. *et al.* The c-Jun NH_2-terminal kinase 3 (*JNK3*) gene: genomic structure, chromosomal assignment, and loss of expression in brain tumors. *J. Hum. Genet.* **46**, 182–187 (2001).
73. Nishina, H. *et al.* Stress-signalling kinase Sek1 protects thymocytes from apoptosis mediated by CD95 and CD3. *Nature* **385**, 350–353 (1997).
74. Tournier, C. *et al.* MKK7 is an essential component of the JNK signal transduction pathway activated by proinflammatory cytokines. *Genes Dev.* **15**, 1419–1426 (2001).
75. Teng, D. H. *et al.* Human mitogen-activated protein kinase kinase 4 as a candidate tumor suppressor. *Cancer Res.* **57**, 4177–4182 (1997).
76. Kim, H. L. *et al.* Mitogen-activated protein kinase kinase 4 metastasis suppressor gene expression is inversely related to histological pattern in advancing human prostatic cancers. *Cancer Res.* **61**, 2833–2837 (2001).
77. Yoshida, B. A. *et al.* Mitogen-activated protein kinase kinase 4/stress-activated protein/Erk kinase 1 (MKK4/SEK1), a prostate cancer metastasis suppressor gene encoded by human chromosome 17. *Cancer Res.* **59**, 5483–5487 (1999).
78. Yamada, S. D. *et al.* Mitogen-activated protein kinase kinase 4 (MKK4) acts as a metastasis suppressor gene in human ovarian carcinoma. *Cancer Res.* **62**, 6717–6723 (2002).
79. Hess, P., Pihan, G., Sawyers, C. L., Flavell, R. A. & Davis, R. J. Survival signaling mediated by c-Jun NH_2-terminal kinase in transformed B lymphoblasts. *Nature Genet.* **32**, 201–205 (2002).
80. Dumas, J. Protein kinase inhibitors: emerging pharmacophores 1997–2000. *Exp. Opin. Ther. Patents* **11**, 405–429 (2001).
An excellent review of the many different kinase inhibitor chemical templates that were identified in the late 1990s.
81. Gray, N. S. *et al.* Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors. *Science* **281**, 533–538 (1998).
82. Zhang, F., Strand, A., Robbins, D., Cobb, M. H. & Goldsmith, E. J. Atomic structure of the MAP kinase ERK2 at 2.3 Å resolution. *Nature* **367**, 704–711 (1994).
83. Wilson, K. P. *et al.* Crystal structure of p38 mitogen-activated protein kinase. *J. Biol. Chem.* **271**, 27696–27700 (1996).
84. Xie, X. *et al.* Crystal structure of JNK3: a kinase implicated in neuronal apoptosis. *Structure* **6**, 983–991 (1998).
The report of the JNK3 crystal structure provided a key tool for the identification of selective JNK inhibitors.
85. Bennett, B. L. *et al.* WO 0112609 (2001).
86. Bain, J., McLauchlan, H., Elliott, M. & Cohen, P. The specificities of protein kinase inhibitors: an update. *Biochem. J.* **371**, 199–204 (2003).
87. Kois, A. *et al.* WO 200246170 (2002).
88. Press release, dated 10/21/2002; see http://www.corporate-ir.net/ireye/ir_site.zhtml?ticker=celg&script=410&layout=9&item_id=346725.
A press release announcing the first Phase I clinical trial of a selective JNK inhibitor.
89. Halazy, S., Church, D., Camps, M. & Gotteland, J. P. WO 0147920 (2001).
90. Arkinstall, S. *et al.* WO 0123379 (2001).
91. Arkinstall, S. *et al.* WO 0123378 (2001).
92. Arkinstall, S. *et al.* WO 0123382 (2001).
93. Salituro, F. G. *et al.* WO 0064872 (2000).
94. Green, J. *et al.* WO 0112621 (2001).
95. Ohkawa, S., Naruo, K., Miwatashi, S., Kimura, H. & Kawamoto, T. WO 2002062792 (2002).
96. Corbett, W. L. & Luk, K.-C. & Mahaney, P. E. WO 0035909 (2000).
97. Luk, K.-C., Mahaney, P. E. & Mischke, S. G. WO 0035906 (2000).
98. Luk, K.-C. & Michoud, C. WO 0035921 (2000).
99. Radcliffe, A. J. *et al.* WO 2003024967 (2003).
100. Oinuma, H., Ohi, N., Sato, N. & Seshimo, H. WO 2002083648 (2002).
101. Graczyk, P. *et al.* WO 2002081475 (2002).
102. Lograsso, P. *et al.* WO 200191749 (2001).
103. Weston, C. R. & Davis, R. J. The JNK signal transduction pathway. *Curr. Opin. Genet. Dev.* **12**, 14–21 (2002).

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